Biochemical Pharmacology, 1963, Vol. 12, pp. 293–302. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Non-classical antimetabolites. XI.*
The bridge principle of specificity with exoalkylating irreversible inhibitors. IV.
Highly selective inhibition of the substrate-identical enzyme from two different tissues

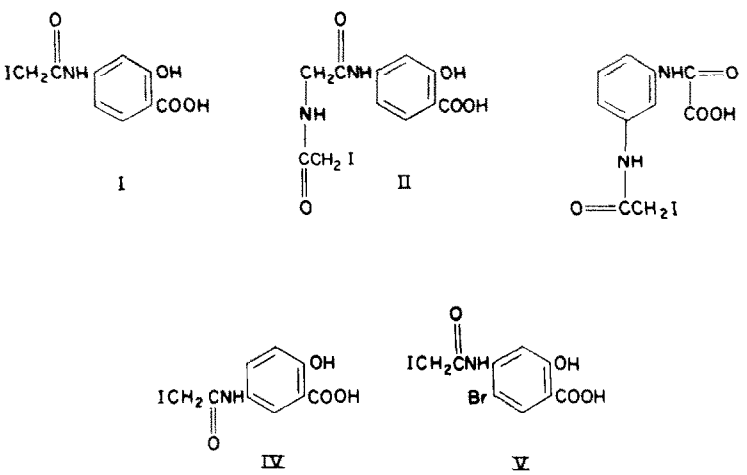
(*Received* 1 *November* 1962; *accepted* 14 *November* 1962)

PREVIOUS papers in this series on a new class of irreversible inhibitors that presumably operate by exoalkylation[1, 2] led to the concept[2] and experimental verification[3, 4] of the bridge principle of specificity: compared to a reversible inhibitor, the exoalkylating type of irreversible inhibitor can have an extra dimension of specificity; this extra specificity is dependent upon the ability of the reversibly-bound inhibitor to bridge to and alkylate a nucleophilic group on the enzyme surface and upon the nucleophilicity of the enzymic group being alkylated.

With the mechanistically closely related enzymes, LDH and GDH,† both facets of the bridge principle have been verified: first, the difference in ability of certain inhibitors to bridge to alkylate an enzyme nucleophilic site;[3, 4] and second, the difference in the nucleophilicity of the enzymic group being alkylated.[5]

Since the enzymic nucleophilic site is presumably in the secondary or tertiary structure of the protein other than the active site,[4] and since the substrate-identical enzyme from two different tissues[6, 7] frequently gives no cross reaction with specific antisera, we suggested[4] that—via the bridge principle—it may be possible to obtain highly selective irreversible inhibitors of the substrate-identical enzyme from two different tissues. That such specific irreversible inhibition is indeed possible has now been demonstrated with LDH from two different tissues and is the subject of this communication.

It has been shown by antisera cross reactions[7, 8] and by amino acid analysis[8] that the lactic dehydrogenases from heart and from skeletal muscle in the same animal are quite different, but that the skeletal muscle LDH's from different species are more similar; also heart LDH's from different species are more similar.[7, 8] The current study was carried out with the commercially available‡ crystalline LDH isolated from rabbit skeletal muscle and LDH isolated from beef heart. Although



* This work was generously supported by Grant CY-5867 of the National Cancer Institute. For the previous paper in this series see Reference 10.

† LDH, lactic dehydrogenase; GDH, glutamic dehydrogenase.

‡ Purchased from Sigma Chemical Co.

these enzymes are from two distinct tissues of two species, these experiments give a first approximation of the selective inhibition that might be obtained.

4-(Iodoacetamido) salicylic acid (I), the standard compound used for irreversible inhibition of LDH and GDH,[1-5] and four related compounds (II–V)[9] were investigated as inhibitors of heart LDH. As reversible inhibitors (Table 1), the compounds varied from equally effective on heart LDH (compound III) to one-sixth as effective (compound II); however, all five compounds gave reversible inhibition of both enzymes. Striking differences in irreversible inhibition were observed. Of the four compounds (I, II, IV, V) that gave irreversible inhibition of muscle LDH, only compound II showed irreversible inhibition of heart LDH, and *the remaining three compounds showed no detectable irreversible inhibition of heart LDH.* Although compound III is the most strongly bound reversibly, it showed no irreversible inhibition with either of the two enzymes (Table 1). Even though no cross-over specificity was noted with these five compounds, as previously observed with LDH and GDH,[3, 4] the fact that II irreversibly inhibits heart LDH makes it probable that a compound irreversibly effective on heart LDH, but ineffective on muscle LDH, could be found by further search; nonetheless

TABLE 1. IRREVERSIBLE INHIBITION OF HEART AND SKELETAL MUSCLE LDH

Compound	Relative halogen reactivity*	Skeletal muscle LDH*		Heart LDH	
		$10^4 \times K_I$†	Relative rate of inactivation	$10^4 \times K_I$†	Relative rate of inactivation§
II	1·00	4·0	1·0‡	25	1·0‡
I	1·12	17†	1·1	45	0
III	1·34	1·3	0	1·2	0
IV	0·78	5·0	0·90	19	0
V	0·98	7·3	1·8	28	0

* Data taken from Reference 2.

† Calculated from the expression $K_I = Km \times I/S$ where I is the concentration of inhibitor giving 50% inhibition in the presence of a rate-saturating concentration of substrate S; K_I is the dissociation constant for the enzyme inhibitor (EI) complex, and Km is the dissociation constant for the enzyme substrate (ES) complex. This method is perhaps as accurate as the reciprocal plot method, and is sufficiently accurate to indicate the relative magnitudes of EI complex formation. In our system, $Km = 2{\cdot}5 \times 10^{-4}$ for skeletal muscle LDH and $0{\cdot}95 \times 10^{-4}$ for heart LDH.

‡ Arbitrary assignments for relative purposes; the absolute values for the two LDH's are not the same.

§ Determined as previously described for muscle LDH except[1, 2, 4] that incubation time was 120 min at 37°.

the current data demonstrate that irreversible specificity for substrate-identical enzymes from different tissues can exist.

If the carry-over of this irreversible specificity for substrate-identical enzymes from different tissues or different cells can be realized in such critical enzymic areas for cell division as (a) purine or pyrimidine biosynthesis or (b) for the folic cofactor area, the potential utility in chemotherapy would be obvious. Such studies in these two areas are continuing in these Laboratories.*

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* The excellent technical assistance of Maureen Vince is gratefully acknowledged.

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Studies of the antagonism of guanethidine by methamphetamine

(*Received* 1 *October* 1962; *accepted* 8 *November* 1962)

GUANETHIDINE, a well-known antihypertensive agent, possesses two distinct properties: (1) blockade of the adrenergic neuron[1] and (2) depletion of catecholamine from heart, spleen, and intestine.[2] The former property has been attributed to its bretylium-like action,[1] *i.e.* the inhibition of release of the neurohumoral agent from the terminals of the adrenergic neuron. The depletion of catecholamine by guanethidine requires the presence of the depleting agent in the tissue.[3] After a single administration of guanethidine, maximal depletion occurs after 4 hr and is maintained for approximately 18 to 24 hr, after which the catecholamine concentration begins to rise. Kuntzman *et al.*[3] reported that, after a single administration, guanethidine is present in tissue for 18 to 24 hr. It was reported recently that the adrenergic neuronal blocking property of guanethidine is antagonized by several sympathomimetic amines[4] and cocaine. This antagonism appears to be competitive in nature.[5] If it is assumed that the antagonism between the sympathomimetic amines and guanethidine is competitive, it may be possible that the depletion of catecholamines by guanthidine and the uptake of guanethidine by tissue are decreased in the presence of sympathomimetic amines. This present investigation demonstrates that in the presence of the sympathomimetic amine, methamphetamine, there is a decrease in the depletion of the catecholamine and in the uptake of guanethidine.

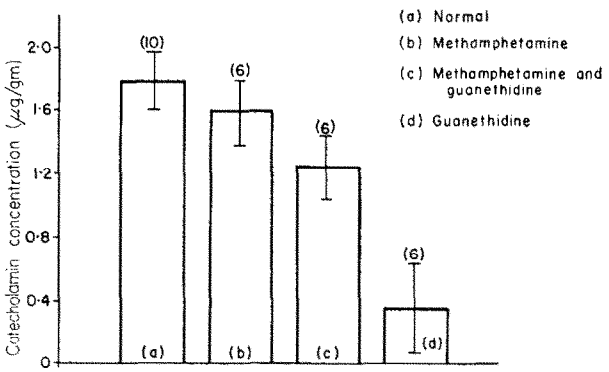

FIG. 1. Levels of catecholamines in hearts of rabbits pretreated for 4 hr with either guanethidine (12·5 mg/kg, i.v.), methamphetamine (20·0 mg/kg, i.p.), or both. Concentration, µg/g tissue ± SD. Numbers in parentheses represent the number of animals used in the experiment.

New Zealand white rabbits, 2·0 to 2·5 kg, of either sex, were used. An aqueous solution of guanethidine was injected (12·5 mg/kg) into the marginal ear vein; methamphetamine was injected intraperitoneally (20·0 mg/kg). When both drugs were used in one animal, guanethidine was injected first and immediately followed by methamphetamine.

The degree of depletion of catecholamine was measured in cardiac tissue from animals pretreated for 4 hr with the various test compounds. The fluorometric analysis of the catecholamine was done